

SUB2

--65. A method of altering expression of a gene that is present in a primary or secondary cell, said method comprising introducing a DNA construct comprising a regulatory region into the genome of said cell by homologous recombination, wherein said regulatory region is inserted into, or replaces all or a portion of, the regulatory region of said gene, thereby producing a primary or a secondary cell in which the expression of said gene is altered.--

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--66. The method of claim 65, wherein the primary or secondary cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

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--67. The method of claim 65, wherein the primary or secondary cell is of mammalian origin.--

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--68. The method of claim 67, wherein the primary or secondary cell is a human cell.--

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--69. The method of claim 65, wherein the gene is selected from the group consisting of genes that encode enzymes, cytokines, hormones, antigens, antibodies, clotting factors, regulatory proteins, transcription proteins, and receptors.--

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(2) --70. The method of claim 69, wherein the gene is selected from the group consisting of the human erythropoietin, human growth hormone, human insulin, and human Factor VIII genes.--

(2) --71. The method of claim 65, wherein expression of the gene is increased.--

(2) --72. The method of claim 65, wherein the DNA construct further comprises a positive selection marker, and the method further comprises the step of selecting for cells comprising said positive selection marker.--

(2) *negative marker* --73. The method of claim 72, wherein the DNA construct further comprises a negative selection marker, and the method further comprises the step of selecting against cells comprising said negative selection marker.--

(2) --74. The method of claim 72, wherein the positive selection marker is the *neo* gene, and cells comprising said positive selection marker are selected by culture in medium comprising G418--

X --75. The method of claim 73, wherein the negative selection marker is the *gpt* gene, and cells comprising said negative selection marker are selected by culture in medium comprising 6-thioxanthine.--

X --76. The method of claim 73, wherein the negative selection marker is the Herpes Simplex Virus thymidine kinase gene, and cells comprising said negative selection marker are selected by culture in medium comprising gancyclovir.--

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CONT'D

--77. A primary or secondary cell produced by the method of claim 65.--

R --78. A clonal cell strain of secondary cells produced by the method of claim 65.--